PRIVILEGED AND CONFIDENTIAL
ATTORNEYS' WORK PRODUCT

Covington & Burling DRAFT -

August 3, 1987

SUMMARY OF DATA ON CAROB BEAN GUM AND EXTRACT

Abstract. Carob bean gum and extract are natural plant derivatives which are used in foods, drugs, and cosmetics. When used as flavors in the manufacture of cigarettes, a typical cigarette will contain less than one milligram of carob bean, or approximately 0.1% of the weight of a cigarette.

Mutagenicity studies comparing cigarette smoke condensate from reference cigarettes to condensate from cigarettes containing 1.5, 3 or 6 times the amount of carob bean gum used in typical commercial cigarettes indicated that carob bean gum does not alter the biological activity of the condensate. Inhalation studies comparing the smoke of reference cigarettes to smoke from cigarettes containing five times the level of carob bean used in a typical commercial cigarette generally indicated no adverse health effects, though in one study equivocal results were obtained.

Studies have shown that carob bean is toxic only at extremely high doses, and oral LD_{50} values for rats, mice, rabbits and hamsters are above 8 grams per kilogram. Tests have shown that carob bean gum is not mutagenic or teratogenic, and a National Toxicology Program bioassay indicated that carob bean gum is not carcinogenic. Carob bean has no adverse cardiovascular or respiratory effects, and it does not appear to be immunotoxic.

Background. Carob bean gum (CAS No. 9000-40-2) is also referred to as St. John's Bread or locust bean gum. Carob bean gum is obtained by removing and processing the endosperm from seeds of the carob tree (Ceratonia siliqua), a large leguminous evergreen which is widely cultivated in the Mediterranean area. Cyprus, Spain, Italy, Greece and Syria are the most important producing areas. Processing of the ground endosperm is accomplished by dispersing the fine powder in boiling water and filtering to remove impurities. The gum

is recovered by evaporating the solution and tray or roll drying (Handbook Food Additives, 1972). The United States imported 5.7 million pounds of carob bean gum in 1975.

Carob bean gum is a neutral galactomannan polymer consisting of a main chain of D-mannose units and a side chain of D-galactose on every fourth or fifth unit (Handbook Food Additives, 1972). As approved by the Food and Drug Administration, carob bean gum should contain not less than 73% galactomannans and not more than 15% water, 8% protein, 5% insoluble material, and 1.2% ash (Food Chemicals Codex, 1981). Carob powder has been reported to contain the following sugars and cyclitols: sucrose, 25-40%, fructose, 3-8%, glucose, 2-6%, pinitol, 5-7% and myo-inositol, 0.5-1% (Baumgartner, 1986).

Carob bean gum is used as a flavor, thickener, binder and stabilizer in food, drug, and cosmetic products. More specifically, carob bean gum is used as a stabilizer in ice cream, sauces, salad dressings, pie fillings, jams and jellies, as a texture modifier in soft cheese, and as a binder in processed meat products. Other manufacturing uses include pharmaceuticals, cosmetics, textiles, paper, ceramics, paints and gum powder (Handbook Food Additives, 1972; Kirk and Othmer, 1966).

Tobacco Uses. Carob bean gum and extract are used as flavors in some commercial cigarettes. Approximately 800,000 pounds were used by cigarette manufacturers in the United States in 1986. A typical usage level is 8.5 lbs. per

11,000 lbs. of tobacco (0.1%). Therefore, a commercial cigarette may contain approximately 0.8 mg of carob bean gum.

Regulatory Status. Carob bean gum and extract are generally recognized as safe (GRAS) food additives by the U.S. Food and Drug Administration. 21 C.F.R. §§ 182.20; 184.1343. Section 184.1343 provides that carob bean gum may be used as a stabilizer and thickener at levels not to exceed the following maximums: baked goods and baking mixes, 0.15%; nonalcoholic beverages and beverage bases, 0.25%; cheeses, 0.8%; gelatins, puddings and fillings, 0.75%; commercial jams and jellies, 0.75%; and all other food categories, 0.5%. Section 182.20 affirms the GRAS status of essential oils, oleoresins, and natural extractives of carob bean.

Acute Toxicity. Low toxicity ratings are supported by reported oral LD_{50} values of more than 8 g/kg in rats, mice, rabbits and hamsters (Bailey and Morgareidge, 1976). Also, in a study performed by the Stanford Research Institute and sponsored by the Food and Drug Administration, carob bean gum produced no unusual or adverse effects when given orally to male Sprague-Dawley rats at either a single dose of 10 g/kg body weight or at an oral dose of 5 g/kg for 5 days (Newell and Maxwell, 1972).

An acute toxicity assessment of carob bean was performed by a single intraperitoneal administration to Sprague-Dawley rats of various doses of a saline solution followed by

a 14 day observation period. The effect of this administration on the rats varied, and an LD_{50} value for males, females, and combined sexes was estimated to be 1260 mg/kg.

Subchronic Studies in Animals. In a subchronic feeding study conducted by the National Toxicology Program (NTP) to determine the concentrations of carob bean gum to use in chronic studies (see Carcinogenicity Studies, below), rats and mice were fed diets containing 0, 6, 300, 12,500, 25,000, 50,000, or 100,000 ppm carob bean gum for 91 days. No compound related effects were noted in clinical observations, necropsies, or histopathological analyses (NTP, 1982).

Groups of 10 male and 10 female rats were fed carob bean gum in their diet at levels of 0%, 1%, 2% or 5% for 90 days. General condition, behaviour, survival, growth, food intake, hematology, blood biochemistry and urinalysis showed no treatment-related differences between test and control groups at any dietary level except that the last glucose level was slightly increased in the 5% group. Gross and microscopic examination did not reveal any pathological changes attributable to ingestion of the gum. An increase in the relative weight of the cecum was observed at the 2% level, but was not considered to have any toxicological importance (Til, 1974).

Groups of newly weaned Sprague-Dawley rats (10/group) were fed a soybean-corn meal diet containing 2% carob bean gum for 36 days. Carob bean gum had no effect on the digestibility of the diet, nor was there any significant effect on growth (Vohra, 1979).

Four groups of five male and five female Beagles were fed 0%, 1%, 5% or 10% of a precooked mixture of carob bean and guar gum (proportions unknown) for 30 weeks. Only at the 10% level were hypermotility and soft, bulky stools observed, which probably had no toxicological significance. At the 10% level digestibility was reduced. No adverse hematological, urinary, gross and histopathological and ophthalmological findings were noted (Cox, 1974).

Groups of day-old broiler chickens (seven per group, breed not specified) were fed a soybean protein-corn based diet containing 2% carob bean gum for 24 days. The dietary intake of the chickens was measured daily for the last week of the experimental period; digestibility of the test diet was calculated from the dry weights of the feed and excreta. The average body weight of chickens and the digestibility of the diet was reduced significantly by the inclusion of carob bean gum in the diet (Vohra, 1979).

Groups of day-old Japanese quail (10 per group) were fed a soybean-meal-corn based diet containing 2% carob bean gum for either 35 or 37 days. The dietary intake of the quail was measured daily for the last week of their experimental period; the digestibility of the diet was calculated from the day weights of the feed and excreta. Average body weight and digestibility of the diet was significantly reduced by inclusion of carob bean gum in the diet (Vohra, 1979).

newly weaned ?

In one study, [weanling] rats were fed a diet containing 50 g/kg carob bean gum for 4 weeks. There was an increase in the weight of the caecal wall and the caecal contents. There was also a significant increase in the activity of the following bacterial enzymes: azo reductase, beta-glucocidase, nitroreductase, nitrate reductase, and urease when compared to controls (Mallett, 1984).

An assessment of the potential of carob bean to induce primary irritation of mucosal membranes was performed by the deposition of 0.1 g of the material into the right lower conjunctival sac of each of six New Zealand White rabbits (3 per sex). Daily observations conducted for a period of 7 days resulted in the classification of carob bean as "mildly irritating."

Subchronic and Chronic Effects in Man. A clinical study of a commercial preparation of carob bean grain as a laxative in doses of "two heaping teaspoonfuls" in 56 patients, some of whom took the preparation regularly for two years, resulted in no untoward effects related to the gastrointestinal tract, and no allergenic reaction (Holbrook, 1951).

Eight infants between the ages of 2.5-5 months were fed meals of sugared milk or sugared milk plus a 1% powder extract from carob bean. Addition of the carob supplement did not alter the duration of the gastrointestinal transit time of the meal. Physiological aerophagy was markedly suppressed by the supplement (Rivier, 1952).

In patients with renal failure, ingestion of 25 g of locust bean gum/day had a laxative effect, decreased high blood pressure, and caused a fall in serum urea, creatinine, and phosphorus by the second week of treatment (Yatzidis, 1979).

Genotoxicity. A variety of tests have indicated that carob bean is not mutagenic. No mutagenic response or alteration in recombination frequency for Saccharomyces cervisiae was observed in the host-mediated assay (Newell and Maxwell, 1972).

Carob bean gum had no adverse effect on either metaphase chromosomes from rat bone marrow or anaphase chromosomes from in vitro cultures of WI-38 (human embryonic lung) cells at any of the dose levels or time periods tested (Newell and Maxwell, 1972).

No consistent responses were observed in the dominant lethal gene test in rats that could be attributed to carob bean gum, and the authors concluded that carob bean gum was not positive in this assay (Newell and Maxwell, 1972).

Carob bean gum was not mutagenic towards Salmonella typhimurium TA1530 or G-46 or towards Saccharomyces cerevisiae D-3 when tested without metabolic activation (Green, 1977). In another test, the mutagenic potential of carob bean was assessed in an in vitro bacterial assay employing a battery of Ames' mutant Salmonella strains capable of detecting both frameshift (strains TA1537, TA1538 and TA98) and base pair

substitution (strains TA1535 and TA100) mutations. No mutagenic activity was detected in a range of doses from 1.0 to 10,000 ug per plate (in triplicate) both in the presence and absence of a metabolic activation system consisting of an Aroclor-induced rat liver homogenate fraction (S9).

A mammalian cell mutagenesis assay was conducted to determine the potential of carob bean to induce forward mutations at the thymidine kinase (TK) locus in L5178Y $TK^{+/-}$ mouse lymphoma cells both with and without an induced mammalian liver metabolic activation system (S9). Carob bean was found to be inactive in inducing significant increases in the mutation frequency at the TK locus, both with and without metabolic activation, at doses exhibiting low to moderate cytotoxicity.

Teratogenic Effects. No teratogenic potential was observed with carob bean gum in studies utilizing rats, mice, hamsters and rabbits (Morgareidge, 1975). Oral intubation of up to 1.3 g/kg of body weight of carob bean gum in anhydrous corn oil to pregnant rats for 10 consecutive days, or up to 1.0 g/kg to pregnant hamsters for 5 consecutive days, produced no clearly discernible effect on nidation or on maternal or fetal survival. The frequency of abnormalities in either soft or skeletal tissues of the test animals was comparable to that occurring spontaneously in the sham-treated controls. In mice, no untoward teratogenic or maternal effects were noted at a level of 280 mg/kg for 10 consecutive days. At 1.3 g/kg, 5 out of 21 mice died. The surviving mice produced normal

litters. In pregnant rabbits, no untoward effects were noted at a level of 196 mg/kg for 13 consecutive days, but at 910 mg/kg a majority of the rabbits died. The surviving rabbits produced normal litters.

Carcinogenicity Studies. In a National Toxicology Program carcinogenesis bioassay (NTP, 1982), carob bean gum at levels of 2.5 and 5% (25,000 ppm or 50,000 ppm) was fed to 50 F344 rats and 50 B6C3F1 mice of either sex for 103 weeks. Control groups consisted of 50 untreated rats and mice of either sex. While the mean body weight of high-dose male mice was slightly lower than that of the control group, no other clinical signs, effects on survival, or histopathologic findings were found to be associated with exposure to carob bean gum. Therefore, it was concluded that, under the conditions of the bioassay, carob bean gum was not carcinogenic.

Cardiovascular and Respiratory Toxicity. The acute effects of carob bean on cardiovascular and respiratory function were assessed by its intravenous administration to lightly-anesthetized young adult male Beagle dogs at doses of 0.4, 0.8, and 2.0 mg/kg. A battery of tests monitored effects on cardiac function, systemic circulatory function, segmental vascular function, and respiratory parameters including rate, tidal volume, and minute volume. No statistically significant changes in cardiovascular or pulmonary function were observed between the control and test animals, and the data indicated no physiological impairment of cardiovascular or pulmonary

function due to the acute intravenous administration of carob bean.

Hepatic Enzyme Induction. The capacity of carob bean to induce hepatic microsomal enzyme activities was assessed following its oral administration at 2500 mg/kg (1/2 maximum tolerated dose) daily for 4 days to mice and rats of both sexes. No effect on hexobarbital-induced sleep times in mice was observed, and neither male nor female rats exhibited increases in relative liver weights, p-nitroanisole-O-demethylase or aniline hydroxylase activities.

Immunotoxicity. A screening test for immunosuppressive potential was conducted by the oral administration of carob bean to male B6C3F1 mice at a dose of 2500 mg/kg (1/2 maximum tolerated dose) daily for 11 days. The animals were sensitized to sheep red blood cells (SRBC) by intraperitoneal injections on the third day of test material administration. A hemagglutination assay performed on the 12th day following the initiation of dosing showed no suppression of the anti-SRBC primary immune response among carob bean-treated mice, suggesting that the substance lacks immunosuppressive potential even at the highest dose levels administered.

Pyrolysis Chemistry Studies. Pure carob powder was pyrolyzed at 700°. Using gas chromatography/mass spectrometry, the following pyrolytic products were identified, though a quantitative analysis was not done. It is not known whether the use of carob bean as a tobacco ingredient would produce the same products.

CAROB POWDER PYROLYSIS PRODUCTS

COMPOUND	COMPOUND
Acetal acetate	4-Hexene-2-one (cis?)
Acetaldehyde	2-Hydroxy-3-methyl-2-cyclopenten-1-one
1-(Acetyloxy)-2-propane	1-Hydroxy-2-propane
Benzene	Methylbenzene
1,2-Benzene-dicarboxylic acid, diethyl ester	2-Methyl-1,3-butadiene
1,3-Butadiene	3-Methyl-3-buten-2-one
Butanal	2-Methylfuran
2,3-Butanedione	5-methyl-2-furancarboxaldehyde
2-Butanone	2-Methylpropanal
2-Butenal	2-Methyl-1-propene
Carbon dioxide	2,3-Pentanedione
1,3-Cyclopentadiene	2-Pentenone
2,5-Dihydrofuran (?)	4-Penten-2-one
3-4-Dihydro-2H-pyran (?)	Phenol
1,3-Dimethylbenzene	2-Propanone
2,5-Dimethylfuran	2-Propenal
Ethylbenzene	1-Propene
2-Ethylfuran	Styrene
Furan	
2-Furancarboxaldehyde	
Hexanal	

Pyrolysis Toxicology Studies. The effect of adding carob bean gum to cigarettes was evaluated by testing the resulting cigarette smoke condensate (CSC) in the Salmonella/microsome (S/M) assay (Ames plate incorporation assay). Carob bean gum was sprayed onto 100% blended tobacco at levels 1.5, 3.0, and 6.0 times those normally used in a commercial cigarette (approximately 1.2, 2.4, and 4.8 mg). Due to the solubility

of carob bean gum, the solutions were sprayed onto filler rather than injected into a cigarette. Solvent-sprayed filler was used as the control. Cigarettes were made from the filler, smoked, and the impaction-trapped CSC was tested in the S/M assay (strains TA98 and TA100) with and without metabolic activation. The results indicated that the addition of carob bean gum to cigarettes, at any of the levels tested, did not alter the S/M activity of the CSC.

A series of inhalation studies were performed comparing the toxicity of reference cigarettes to cigarettes to which approximately 13,000 ppm carob bean by weight of the tobacco (approximately 13 mg) had been added as a component of a compounded flavorant mixture. An acute inhalation study was performed in order to establish maximally-tolerated exposure levels for subsequent subchronic studies designed to compare the toxicity of carob-containing cigarettes to reference cigarettes. Groups of six female B6C3F1 mice or six male and six female Fischer 344 rats were exposed to the following low dose regimen in a one-day exposure: a standard two-second 35 mL puff diluted to 10% smoke concentration, thirty seconds smoke alternating with thirty seconds air over eight minutes, eight puffs/exposure, eight minute exposure alternating with an eight minute rest, nine exposures per day. High dose groups of six animals each were exposed to similar puffs at 10% smoke concentration, thirty seconds smoke alternating with thirty seconds air over eight minutes, eight puffs/exposure,

two consecutive eight minute exposures alternating with an eight minute rest, twelve exposures per day. Sham-exposed (no cigarette) controls were treated simultaneously on another smoking machine. The animals exposed to smoke from both carob-containing and reference cigarettes exhibited similar survival rates, carboxyhemoglobin levels, body weight changes, and general physical appearance.

Mice B6C3F1 exposed subchronically (5 days/week for 6 weeks with 31.5 minutes per day total exposure to smoke and 130 minutes per day in smoke chamber) to the smoke of reference or carob bean-containing cigarettes (13,000 ppm) were evaluated for a variety of hematological, clinical chemistry, histological, physical, and bronchioalveolar lavage parameters. Body weight, lung weight, serum chemistry or hematology values were the same for the test and reference groups.

A 90-day subchronic inhalation study was conducted to compare the toxicity of reference cigarettes to cigarettes to which approximately 13,000 ppm carob bean by weight of tobacco (approximately 13 mg) had been added as a component of a compounded flavorant mixture. Nose-only exposures of male and female Fischer 344 rats were performed 5 days/week for 13 weeks, followed by evaluation of an array of physical, clinical chemistry, histological, and bronchioalveolar lavage fluid parameters. No significant differences were noted between the responses of rats exposed to reference cigarette smoke or to the smoke of carob bean-containing cigarettes. An increased

incidence of adenomatous hyperplasia was observed in females exposed to a carob bean-containing cigarette (50% incidence; 5/10) relative to the reference cigarette-exposed female group (10% incidence; 1/10), but not in male rats (10% incidence for both cigarette types; 1/10). This information was insufficient to allow the trend to be statistically evaluated. However, adenomatous hyperplasia has consistently been observed among smoke-exposed animals with variable incidence, i.e., 0-100% incidence among females and 0-70% incidence among males exposed to reference cigarettes or to test cigarettes which do not contain carob as compared to a 30-67% incidence among females and 10-30% incidence among males exposed to carob bean-containing cigarette smoke. Thus, there is no indication that the carob bean component of the flavorant recipe was related to these observations.

REFERENCES

1. Bailey, D., K. Morgareidge, Comparative Acute Oral Toxicity of 12 Food-Grade Gums in the Mouse, Rat, Hamster and Rabbit, Fd Drug Res. Labs. Papers, No. 124 (1976).

2. Baumgartner, S., R.Genner, R. Amado, H. Neukom, Isolation and Identification of Cyclitols in Carob Pod (Ceratonia Siligna L.), J. Agric. Food Chem. 34 827 (1986).

3. Cox, G.E., D.E. Baily, K. Morgareidge, Subacute feeding in dogs with a precooked gum blend, unpublished report from the Food and Drugs Labs, Inc., submitted to the World Health Organization by Hercules, B. V. (1974).

4. Food Chemicals Codex (3d ed. 1981).

5. Green, S., Present and future uses of Mutagenicity tests for assessment of the safety of food additives, J. Envir. Path. Toxicol. 1 49 (1977).

6. Handbook of Food Additives, T. Furia, ed., 295-359 (1972).

7. Holbrook, A.A., The behaviour of carob bean in the gastrointestinal tract of man, A. J. Dig. Dis. 18 24-28 (1951).

8. Kirk, R.E., D.F. Othmer, eds., Encyclopedia of Chemical Technology 10 748-752 (2d ed. 1966).

9. Mallett, A. K., A. Wise, I. R. Rowland, Hydrocolloid Food Additives and Rat Caecal Microbial Enzyme Activities, Fd. Chem. Toxic. 22 415 (1984).

10. Morgareidge, K., Teratological evaluation of FDA-71-14 (Carob bean gum), Unpublished report from the Food and Drug Research Labs, Inc., Submitted to WHO by Hercules, BV, Cited in WHO Fd Add. Ser. 8 38 (1975).

11. Newell, G.W., W.A. Maxwell, Study of Mutagenic Effects of Locust Bean Gum (FDA-71-14), Stanford Research Institute, U.S. National Technical Information Service, PB-221819 (1972).

12. National Toxicology Program (NTP), Carcinogenesis Bioassay of Locust Bean Gum (CAS No. 9000-40-2) in F344 Rats and B6C3F1 Mice (Feed Study), NTP TR221, NTP-80-66, DHHS NIH Publication No. 82-1777, Carcinogenesis Testing Program,

National Institute of Environmental Health Sciences, National Institutes of Health, Bethesda, MD (1982).

13. Rivier, C., Recherches sur le mode d'action du Nestargel, Schweiz. Mediz. 82 256 (1952).

14. Til, H.P., M. Th. Spanjers, A.P. de Groot, Subchronic study with locust bean gum in rats, Unpublished report from Centraal Instituut voor Voedingsonderzoek TNO submitted to the World Health Organization by Hercules, B. V and Institut Europeen des Industires de la Gomme de Caroube (1974).

15. Vohra, P., G. Shariff, F.H. Kratzer, Growth inhibitory effect of some gums and pectin for Tribolium castaneum larvae, chickens and Japanese quail, Nutr. Rep. Internatl. 19(4) 463-469 (1979).

16. Yatzidis, H., D. Koutsicos, P. Digenis, Newer oral sorbents in uremia, Clin. Nephrol. 11(2) 105-106 (1979).